SMALL INTESTINE: ABSORPTIVE CELLS

The ultrastructures of small intestinal enterocytes, which line the villi and are continually renewed, mirror their main tasks in the absorption of digested food. Panels A and B show the apical cell domain of absorptive cells with the prominent brush border made up of numerous densely packed microvilli, which are the sites of final digestion and uptake of nutrients. Microvilli exist in an approximate number of 3,000 per cell, which greatly increases the luminal cell surface. They measure 1–2 μm in length and contain a filamentous core, composed of actin filaments, crosslinked by fimbrin and villin, and attached to the plasma membrane by myosin I and calmodulin. The rootlets of the filament bundles project into the terminal web located beneath the brush border (tw). They are interconnected by intestine-specific spectrin, attach to cytokeratin intermediate filaments of the terminal web, and are part of the apical cytoskeletal apparatus that is responsible for maintaining the upright positions of the microvilli and the overall organization of the brush border (cf. also Fig. 88). Components of the terminal web are associated with the junctional complex. In particular, actin filaments connected with the belt desmosome in the middle part of the junctional complex (panel B-2; cf. also Fig. 98) contribute essentially to the cells-cells expanding motion system. This is responsible for changes of the diameter of the apical cell domains, leading to a tilting of the brush border microvilli, which facilitates contact with the digested nutrients and supports absorption.

The luminal cell surface is lined by a 10 nm thick plasma membrane, clearly visible in panel B as a distinct three-lamellar structure covered by the fuzzy, filamentous network of the glycocalyx (cf. Fig. 94). Intramembraneous enzymes are responsible for the final breakdown of oligosaccharides and oligopeptides. The products, such as amino acids, di- and tripeptides, and sugars, are transported across the plasma membrane, which involves specific membrane channels and carrier systems.

Brush border enzymes and other membrane constituents have to be continually renewed and are inserted into the plasma membrane at areas located between the microvilli. These are the only membrane sites of the apical surface accessible for fusion and budding events and, from here, membrane invaginations deeply extend into the terminal web region. They appear as pleiomorphic compartments and apical tubules close to the microvilli rootlets (cf. Fig. 88, panels A, D and Fig. 98, panel B). Their membranes exhibit lipid microdomains different from those of the microvilli membranes and are considered to function as a membrane reservoir for adaptative changes at the apical cell surface.

Regular absorption requires a clear cell polarity with distinct boundaries between apical and basolateral cell surfaces, which are constituted by tight junctions forming the most apical zone of the junctional complex that connects adjacent cells. Panel A shows the apical part of an absorptive cell with numerous mitochondria, lysosomes (Ly), and the Golgi apparatus in typical supranuclear position (Golgi). Bars indicate the profiles of the junctional complex, which is shown at higher magnification in panel B. Close to the apical cell surface, tight junctions form an occluding belt, which defines cell polarity, seals the intercellular spaces, and controls the intercellular passage of substances (1; zonula occludens). The second and the third parts of the junctional complex are built up by adhering junctions forming a belt desmosome closely below the tight junctions (2; zonula adhaerens) and an additional circle of spot desmosomes (3; maculae adhaerentes). At more basal regions, adjacent cells are often interlocked by extended interdigitations (panel C).

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Magnification: ×12,500 (**A**), ×57,000 (**B**, **C**)

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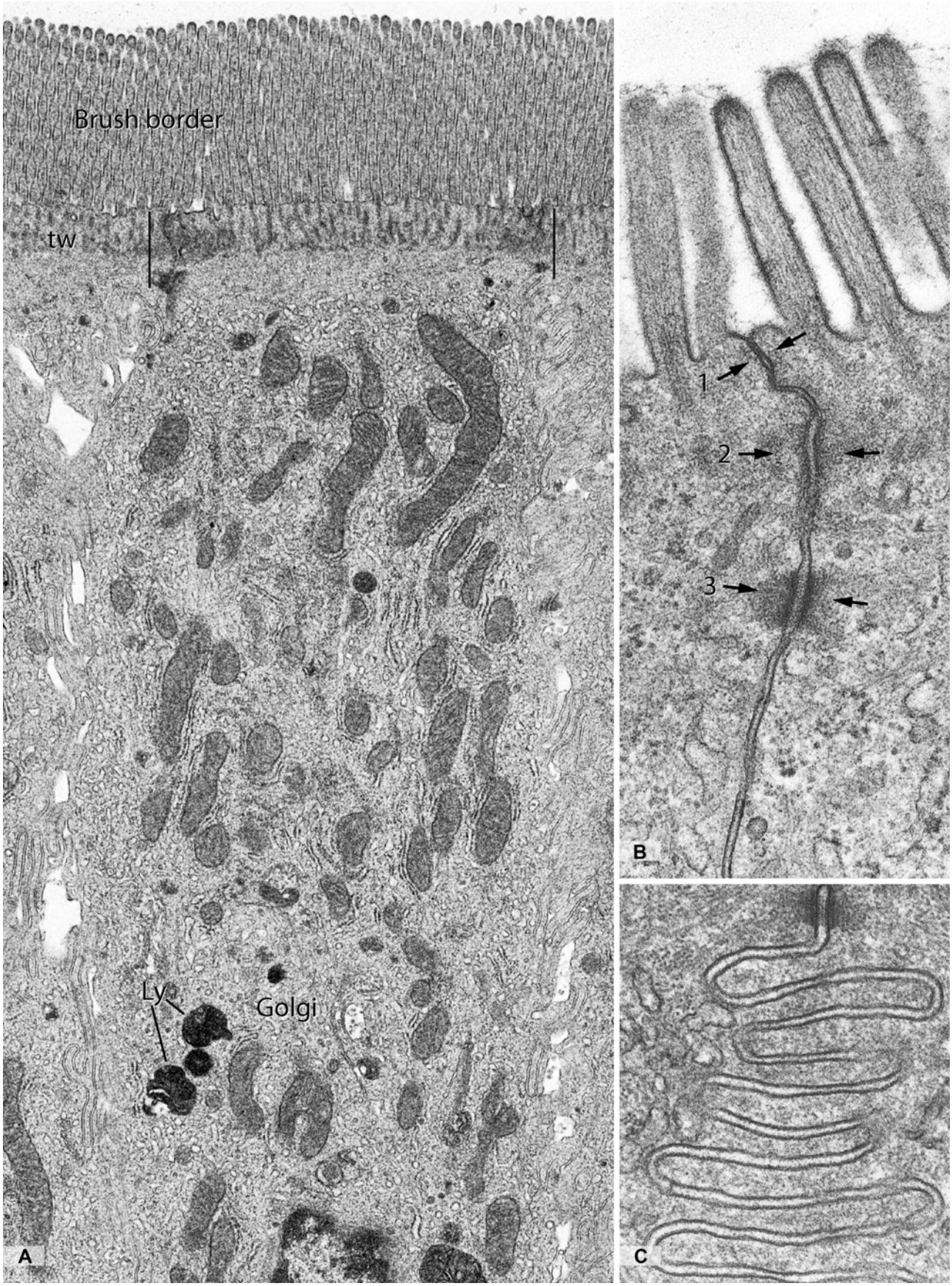
DOI 10.1007/978-3-7091-1830-6_15, © Springer-Verlag Vienna 2015


Brush border
tw
Ly
Golgi
A
1
2
3
B
C

SMALL INTESTINE: PATHWAY OF LIPIDS

Through the absorptive enterocytes, digested nutrients cross from the lumen of the gut to the connective tissue underlying the epithelium. After ingestion and specific processing by the absorptive cells (cf. Fig. 128), nutrient constituents are transported to the basolateral cell surfaces, where they again leave the cells and enter blood or lymph capillaries to be distributed in the body. A particular route across the absorptive cells is traveled by lipids, which in the majority are packed into lipoprotein particles, secreted by exocytosis, and moved into the lumen of lymphatics. Some stages of the transcellular and extracellular lipid pathways can readily be followed under the electron microscope.

In the gut lumen during fat breakdown, mixed micelles containing bile salts and the main products of fat digestion are assembled and have immediate access to the brush border through movements of villi and microvilli. Free fatty acids and monoglycerides liberated from the micelles diffuse into the microvilli and associate with fatty acid-binding proteins, to be transported into the apical cytoplasm. In the smooth endoplasmic reticulum, triglycerides and other lipids are resynthesized and packaged into lipoprotein particles (LPs). Subsequently exported out of the endoplasmic reticulum and transported to the Golgi apparatus, they are glycosylated and packaged into vesicles to be transported to the basolateral cell surfaces. Via exocytosis, they leave the cell into the intercellular spaces. Particularly large LPs, which are known as chylomicrons, are formed during postprandial lipid absorption. However, LPs are not only formed after intake of food. Lipids, mainly derived from the bile and shedded cells, are also absorbed during starvation. The LPs formed during starvation measure 50–80 nm in diameter and belong to the class of very low density lipoproteins (VLDLs; cf. Figs. 124 and 125).

Panels A–D show the intra- (A) and extracellular (B–D) pathways of the small intestinal VLDL particles in the mucosa of a starving rat. VLDL particles are visible in all compartments along the secretory pathway. They are particularly prominent in the dilated cisterns of the Golgi apparatus stacks (arrows in panel A), in which they are taken up after export out of the endoplasmic reticulum. Golgi cisternae are the sites of VLDL glycosylation. In the Golgi apparatus, VLDL particles are packed into large carrier vesicles for transport to the lateral cell surface. One of the carriers is shown in panel A at the left side of the Golgi stack. Secretion of VLDLs is known to occur via exocytosis. Extracellularly, VLDL particles are apparent in the dilated region of the intercellular space (arrowheads in panel B) located between extended cell-cell interdigitations and are accumulated in the connective tissue of the mucosal lamina propria (arrowheads in panel C). They do not enter the blood but move into the lumen of the lymphatics. This is shown in panel D. Here, lipoprotein particles appear negatively stained because all extracellular fluid of the lamina propria of this rat small intestinal mucosa contains peroxidase, applied intravenously in connection with transport experiments, and visualized by oxidation of diaminobenzidine. The lumina of blood capillaries are devoid of lipoprotein particles (panel C).

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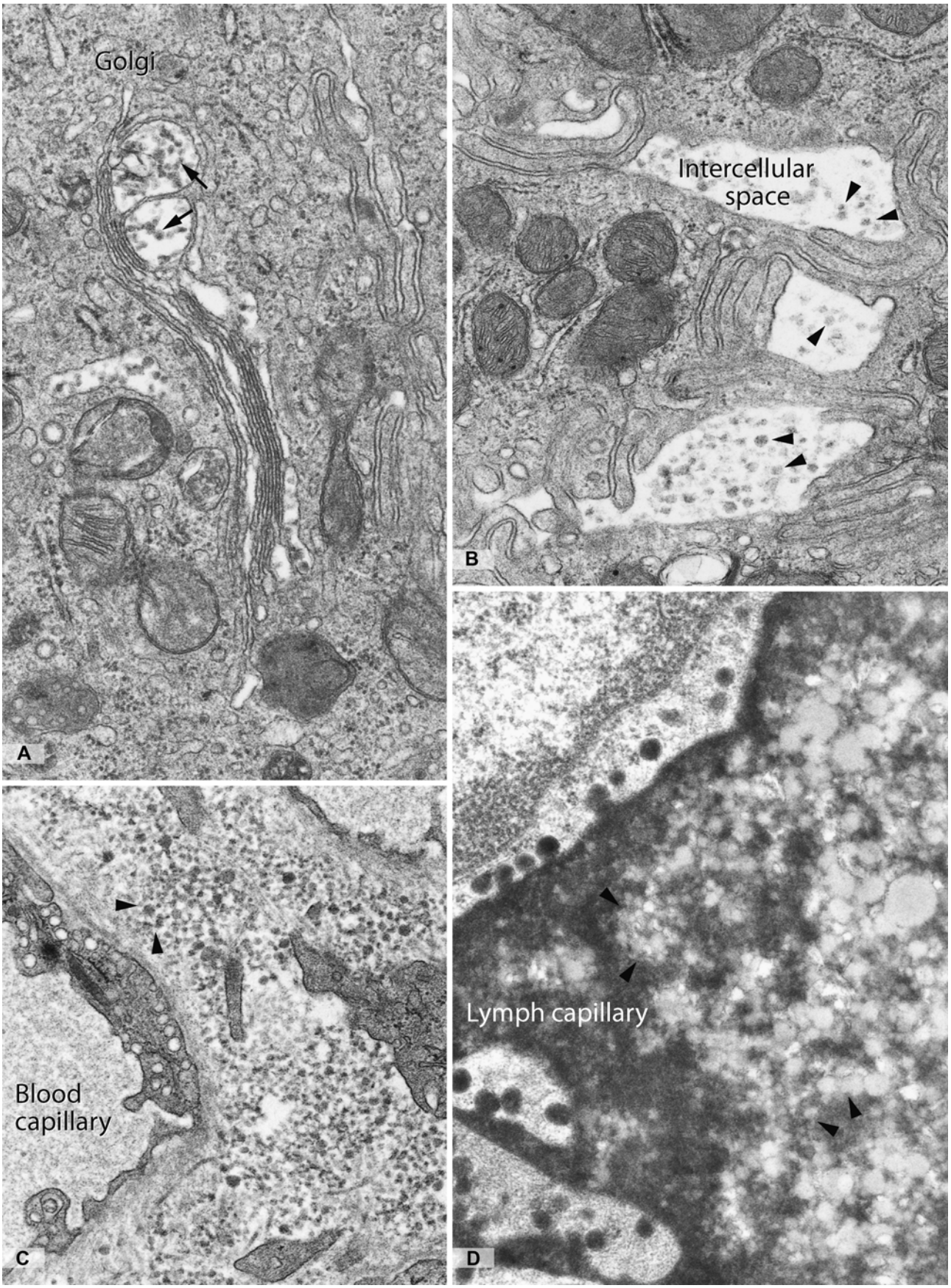
Magnifications: ×33,500 (**A**), ×28,000 (**B**), ×18,300 (**C**), ×36,500 (**D**)


Golgi
A
Intercellular space
B
Blood capillary
C
Lymph capillary
D

RENAL PROXIMAL TUBULE: A REABSORPTION PLANT

The plasma ultrafiltrate generated in the glomeruli is extensively modified and concentrated along the renal tubule. In the proximal tubules, some 70 % of the filtered water, glucose, ions, vitamins, low molecular mass proteins, drugs, and other substances are reabsorbed to the blood or degraded in lysosomes of kidney epithelia.

Proximal tubules have function-related structural specializations: an apical brush border (cf. Fig. 128) for absorption from the lumen and a basal membrane labyrinth (cf. Fig. 104) for excretion in the extracellular space. These specializations are important for transcellular transport because they provide vast membrane surfaces for receptors, carriers, and transporters. The apical and basolateral plasma membrane domains are functionally different and thereby establish the epithelial asymmetry. Besides the transcellular route, a paracellular route exists for water and ions, which pass the tight junctions by osmosis.

In proximal tubules, an apical endocytic apparatus occupies most of the apical cytoplasm (panel A), which consists of endocytic vesicles (arrow in B) and dense tubules (arrowheads in B). The sections shown in panels A and B have been stained to preferentially contrast the glycocalyx of the plasma membrane and glycans of the apical endocytic apparatus. Various substances that undergo receptor-mediated endocytosis via coated pits situated between the bases of microvilli are routed to this endocytic apparatus and from there to lysosomes for degradation. The dense tubules represent structures for membrane recycling.

In proximal tubules, two major endocytic receptors have been identified: megalin and cubilin. In panel C, megalin, as detected in an ultrathin frozen section by immunogold labeling, is present in brush border microvilli and the apical endocytic apparatus. The lateral plasma membrane (PM) is unlabeled. Megalin was initially described as the Heymann nephritis antigen gp 330. Many organs and cell types (e.g., inner ear, type II alveolar epithelial cells, thyroid, choroid plexus) in addition to kidney express megalin. Megalin belongs to the low density lipoprotein receptor family. It is a single-spanning type 1 membrane glycoprotein with a molecular mass of about 600 kDa and is structurally different from cubilin, which is a 460 kDa peripheral membrane glycoprotein. Both receptors contain a large extracellular domain, which functions in ligand binding, and they form a dual receptor complex. Because megalin binds a multitude of ligands, it is regarded as a scavenger receptor. Megalin is involved in vitamin homeostasis; it binds at least three vitamin-binding proteins: transcobalmin-vitamin B_{12}, retinol-binding protein, and vitamin D-binding protein. Other ligands for megalin involve different carrier proteins (e.g., albumin, lactoferrin, transthyretin), various lipoproteins, hormones (e.g., insulin, parathyroid hormone, epidermal growth factor), enzymes, and enzyme inhibitors. Polybasic drugs such as the antifibrinolytic aprotinin, polymyxin B, and aminoglycosides such as gentamycin are reabsorbed by megalin and accumulate in lysosomes of proximal tubules. This is probably the basis for the known nephrotoxicity of polymyxin B and gentamycin.

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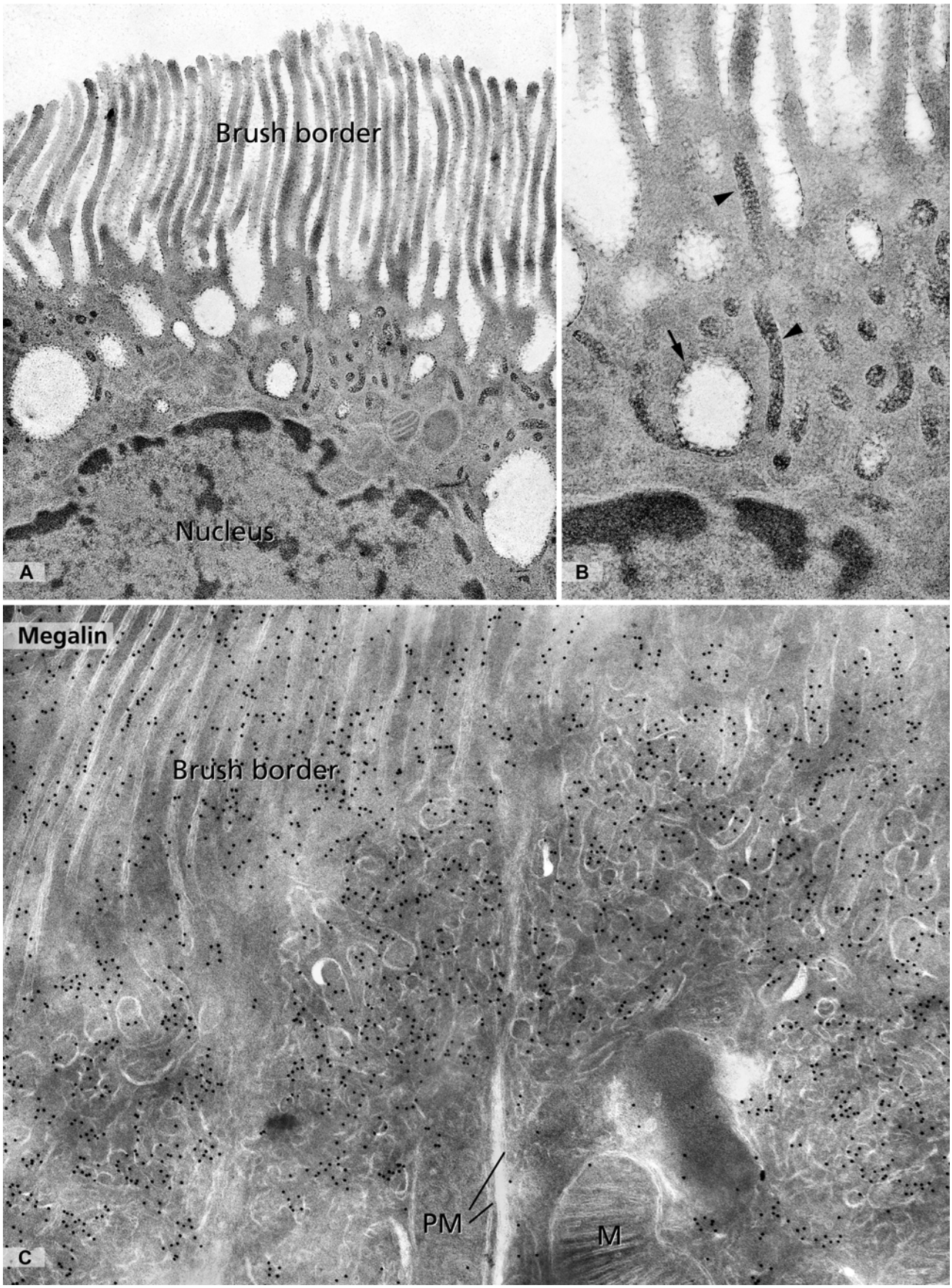
Magnification: ×20,000 (**A**); ×50,500 (**B**); ×45,000 (**C**)


Brush border
Nucleus
A
B
Megalin
Brush border
PM
M
C

PARATHYROID HORMONE RESPONSE OF RENAL PROXIMAL TUBULES

The epithelia lining the proximal tubules must adapt to varying functional demands imposed by changes in the water-electrolyte homeostasis. Experimental studies in which animals were adapted to certain salt diets or subjected to hormone treatment have provided a mechanistic insight into these adaptive changes and will be illustrated by the example of phosphate ion reabsorption in proximal tubular epithelia.

Phosphate ions (Pi) are filtered by the glomeruli, and approximately 80 % of phosphate is reabsorbed in the proximal tubules. Reabsorption occurs through the brush border membrane and requires the presence of sodium ions. Among the three types of Na/Pi cotransporters, the Na/Pi-cotransporter NaPi-IIa has been shown to be the most important one for phosphate ion reabsorption in the proximal tubules. It is a polytope membrane glycoprotein with eight transmembrane domains, four extracellular loops, and three intracellular loops. Intracellular loop 1 and extracellular loop 3 are involved in Na/Pi-cotransport. The cotransporter is found exclusively in the brush border of proximal tubules and its amount in this location determines the capacity for the reabsorption of phosphate ions. NaPi-IIa cotransporter becomes rapidly downregulated in response to parathyroid hormone, which results in inhibition of phosphate ion reabsorption. Quantitative electron microscopy and immunoelectron microscopy revealed the steps resulting in the parathyroid hormone-induced NaPi-IIa downregulation. The brush border membrane became greatly depleted of cotransporter, which accumulated in the apical endocytic apparatus through an increased rate of endocytosis. This resulted in an enlargement of the apical endocytic apparatus. Panel A illustrates the steady state situation, and panel B shows the acute changes in the apical tubulovesicules in response to parathyroid hormone treatment. The difference in the extent of the apical endocytic apparatus is indicated by bars, and the greater abundance of endocytic structures, in particular of the dense tubules, is clearly visible in panel B. This accumulation of the NaPi-IIa cotransporter is transient and followed by its degradation in lysosomes, which resulted in its rapid downregulation. It could also be shown that the lysosomal delivery of the cotransporter is dependent on a taxol-sensitive apical-to-basal rearrangement of microtubules.

In human genetic disorders that result in renal phosphate wasting, the expression of the NaPi-IIa cotransporter seems to be decreased by serum factors such as elevated concentrations of fibroblast growth factor-23.

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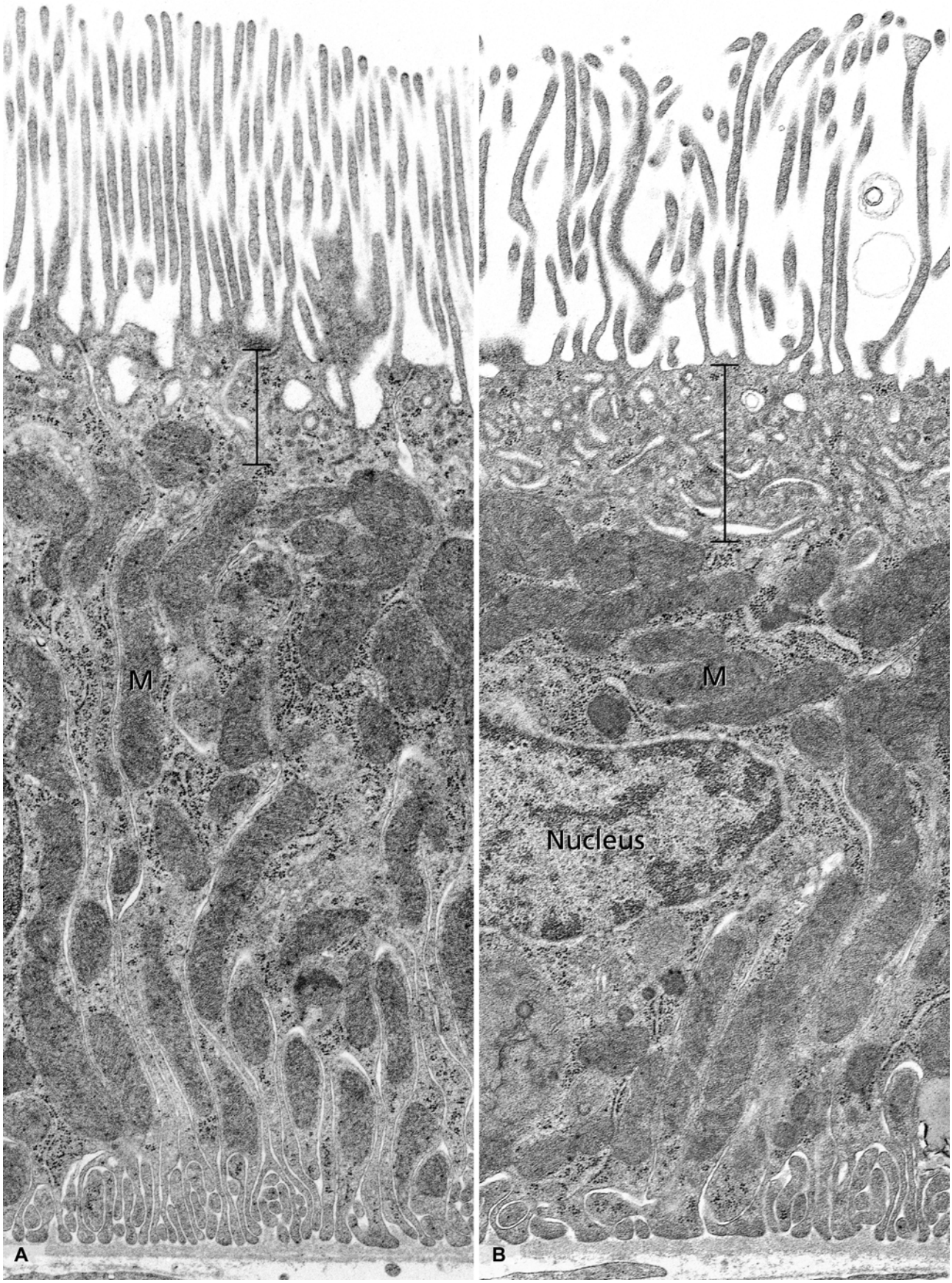
Magnification: ×42,000 (**A**, **B**)


M
M
Nucleus
A
B